Analysis of *Shewanella oneidensis* Membrane Protein Expression in Response to Electron Acceptor Availability

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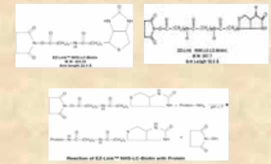
Introduction

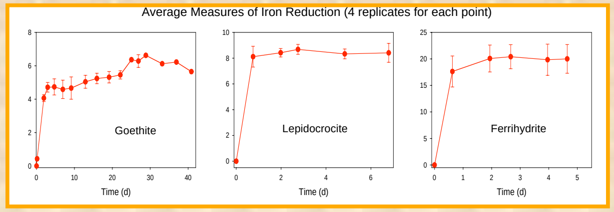
Shewanella oneidensis MR-1, a gram negative metal-reducing bacterium, can utilize a large number of electron acceptors. In the natural environment, *S. oneidensis* utilizes insoluble metal oxides as well as soluble terminal electron acceptors. The purpose of this ERSP project is to identify differentially expressed proteins associated with the membranes of *S. oneidensis* MR-1 cells grown with different electron acceptors, including insoluble metal oxides. We hypothesize that through the use of surface labeling, subcellular fractionation, and a combination of proteome analysis tools, proteins involved in the reduction of different terminal electron acceptors will be elucidated. We are comparing the protein profiles from cells grown with the soluble electron acceptors oxygen and fumarate and with those from cells grown with the insoluble iron oxides goethite, ferrihydrite and lepidocrocite. Comparison of the cell surface proteins isolated from cells grown with oxygen or anaerobically with fumarate revealed an increase in the abundance of over 25 proteins in anaerobic cells, including agglutination protein and flagellin proteins along with the several hypothetical proteins. In addition, the surface protein composition of cells grown with the insoluble iron oxides varies considerably from the protein composition observed with either soluble electron acceptor as well as between the different insoluble acceptors.

S. oneidensis MR-1 cells grown under aerobic or anaerobic conditions with fumarate or with solid iron oxides (goethite, lepidocrocite, or ferrihydrite) were labeled with a 50:50 mixture of EZ-Link Sulfo-NHS-LC-Biotin and EZ-Link Sulfo-NHS-LC-LC-Biotin. Live-Dead staining showed that a majority of the cells were viable after labeling. After sonication to lyse the labeled cells, membranes were isolated by differential centrifugation. Membrane proteins were released using Zwittergent and biotinylated proteins were collected by affinity chromatography with monomeric avidin. Those proteins were separated and identified by two-dimensional electrophoresis with tryptic peptide mass analysis and by two-dimensional liquid chromatography with inline tandem mass spectrometry.

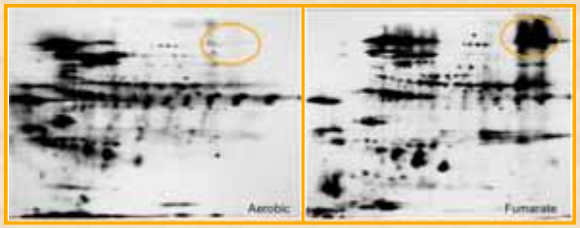


Live/Dead kit, Molecular probes; Syto9-Green fluorescence=Live cells ; Propidium iodide-Red fluorescence=Dead cells

Vials containing goethite, lepidocrocite, or ferrihydrite and Shewanella Federation defined medium were inoculated with *S. oneidensis* MR-1 in late-log phase at an initial density of ~3X10^9 cells/ml and incubated at 30 ºC in the dark on a roller drum. Goethite cultures were sampled at 14 and 28 days, lepidocrocite cultures at 3 and 7 days, and ferrihydrite cultures at 2.5 and 5 days. Cultures were sampled when maximum iron reduction was observed (note the color difference in bottles incubated with *S. oneidensis* in figure to the left; quantitative evidence of the reduction of Fe^{+3} to Fe^{+2} is shown graphically below). Cells were incubated with biotin reagents for 30 min before harvesting. Labeled membranes were released from the iron oxides by incubating with EDTA, $MgCl_2$, and Brij-58 followed by sonication.



Silver stained 2DE patterns of proteins from whole cell lysates of MR-1 grown with different iron oxides (below) indicate differential protein expression. Western blots of the same protein samples, revealing the surface membrane proteins (below silver stained images) also reveal differential protein expression. The identification of proteins in these preparations by 2DLC/MS-MS is in progress.

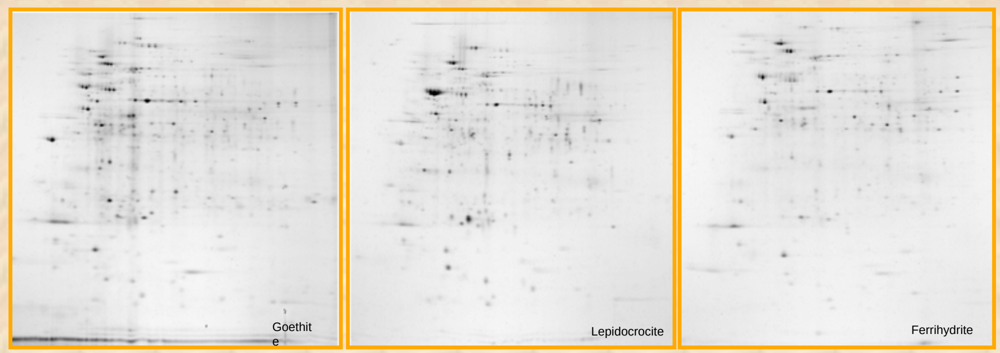


Locus	% Coverage ASO1330 (Anaerobic) LTQ Run 1	% Coverage ASO1330 (Anaerobic) LTQ Run 2	% Coverage ASO1219 (Aerobic) LTQ Run 1	% Coverage ASO1219 (Aerobic) LTQ Run 2	Annotation
g|-7586787|nGITSSbM50PYOOAgdwc5l4y44kc||	22.9	47.9			hemolysin protein putative
g|-7587156|iHzKMUzfKs7bvIqDn3Y24i+IMaw||	29.9	25.4			Hypothetical protein
g|-7587205|9PMS5vHaZNS7saoKd0mcezg3DWg||	25.6	23.9			adhesion protein putative
g|-7587828|SYYhhbql0c+WnGpcc7ILkszO6CU||	24.1	18.4			hypothetical protein
g|-7588027|eEvvuzmD5nZnmT34vOTmm3028JQ||	34.7	36.9			outer membrane protein
g|-7588029|QmkwSXKTKBEmQQ6vHZzaUpK/Yq8||	32.6	40.2			anaerobic dimethyl sulfoxide reductase B subunit
g|-7588028|VOkmEdeCfI5QOJmnfUpiWv0SW+A||	42.1	41.5			anaerobic dimethyl sulfoxide reductase A subunit
g|-7588588|DEQkTtaX8YiKTv4nHotgY2maNnI||	22.9	11.9			conserved hypothetical protein TIGR00103
g|-7588733|aHmbZinKROREyKsXMMhP1D3YhwQ||	27.9	18			cytochrome c551 peroxidase
g|-7588822|1lUplh0+JpSX5ol9D2X8vVS4UvY||	32	56.5			16 kDa heat shock protein A
g|-7589456|3L6pUSpYMxKA2Wy71CJu4yc0KkA||	29.2	18.2			RNA pseudouridylate synthase family protein
g|-7590161|cNKRvJ3kHXgkX10BAKg9MNEaVWU||	29.5	17.8			universal stress protein family
g|-7590780|Fe7N/U7iWe3RCrqlNAUgwn0lpXg||	59.5	59.5			conserved hypothetical protein
g|-7590952|hxGi9nT6MSaxZ0M4tDvx7K3wx8E||	30.2	39.7			formate dehydrogenase iron-sulfur subunit
g|-7591023|lKJtlLGexZj04oJzcVRzQkXQzMQ||	28.5	19.9			hypothetical protein
g|-7595606|saQLuWuuWQFgeEKEdEXF2hT6FLs||	25.6	25.6			putative lipoprotein, putative
g|-7588388|Ybwj1WNNMzOWrJ32C+58TrCaZjE||	89	86.3	12.8	26.9	phage shock protein A
g|-7588360|juQUzzwPI95S7Qx/V83xB2dVr+Q||	60.8	58.2	20.8	19.3	decaheme cytochrome c
g|-7586817|2r/d2okOUjR/CayBIcv4V73ot54||	56.2	52	27.4	20.3	general secretion pathway protein D
g|-7588410|8BKFZzlgGyf2t3Dj9sp/IyE91CI||	46.7	46.5	8.4	16.1	TPR domain protein
g|-7589024|fZlh3dAnfCKIWYRLbTWhrWIs2Sc||	59.9	72.9	12.3	23.2	transcriptional regulator RpiR family
g|-7589127|QKIsZZQKzomLowXOufxdU7Hrpi0||	37.5	35	12.6	12.7	conserved hypothetical protein
g|-7589139|VYjlipzH4+8l+ok2dCezCMcq1dE||	29.7	31.4	5.1	4	PqiB family protein
g|-7589747|Bz30X54huOSiQDa+G0Gp0yte58o||	63.2	57	20.6	21.7	flagellin
g|-7589748|S5wTn68D8e1EVyZpirQlgVa/JUM||	65.6	40.7	20.5	16.1	flagellin
g|-7590245|9UvuZ9q3CIeD2/JvfnHgsr722jI||	91.3	87.3	20.1	30	conserved hypothetical protein
g|-7590377|9AfLXGA4ho+XVIAjWuQtL1d2iaA||	50.6	63.4	26	20	outer membrane protein TolC
g|-7597241|nFLC623jMPjPpRVEx5P6SSuFCPk||	41.5	41.5	10.8	7	conserved hypothetical protein
g|-7590772|MZAFbI3DMulVps91xX6cKrrGkVI||	52.9	52	18	18.3	agglutination protein
g|-7597163|iv55QzSAobQkDlwMQo0htNTykYQ||	8.3	29.5	61	57.8	Vulpibactin outer membrane receptor precursor, putative
g|-7596367|FVxMqj4X5eQKv/QMU6xe6SmptEU||	58.8	52.6	56.6	57.7	Conserved hypothetical protein
g|-7596491|qdQ/H4wkcK0SveWZBj0A5tW5Gmw||	72.1	68.6	75.6	69.1	General diffusion Gram-negative porins, putative
g|-7591172|MwqazFRI9DW5VF/B6MzwO0pLAWg||	34.5	62.7	33.8	54.2	ATP synthase F1 epsilon subunit
g|-7591173|SgE7Q+PNR9/pscj20lXgFGmFoXw||	70.2	82.1	59.6	67.4	ATP synthase F1 beta subunit
g|-7591174|i3vGpQ9cGUXkdSj73xnD4AAQM64||	60.1	62.9	44.4	59.4	ATP synthase F1 gamma subunit
g|-7591175|wxx1+Rvv9hkdqKyLoiiJNDeMb4E||	67.3	69.6	55.2	55.8	ATP synthase F1 alpha subunit
g|-7591177|6/GNNbaCw4I5wXC0W+mfVjDsGuk||	58.3	57.7	40.4	41.7	ATP synthase F0 B subunit

Surface Membrane Proteins from MR-1 Grown Aerobically or Anaerobically with Fumarate:

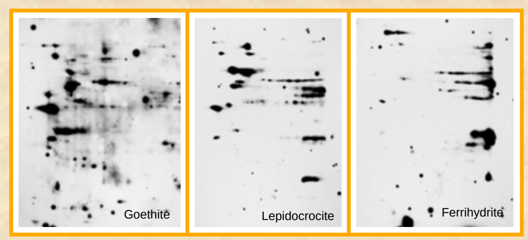
Aliquots of biotinylated proteins from the same preparations were separated by 2DE (at ANL) and by 2D liquid chromatography (at ORNL) and protein identifications were made by tandem mass spectrometry. In 2DE gels, proteins were detected by Western blotting using neutravidin (images shown) and, in replicate gels, by silver staining (data not shown). Proteins were identified by tryptic peptide mass analysis using nanoLC coupled with tandem mass spectrometry using an ESI QStar XL quadrupole time-of-flight mass spectrometer. The images shown are oriented with high molecular weight proteins at the top and low molecular weight proteins at the bottom, acidic proteins to the left and basic proteins to the right. The area circled corresponds to a protein significantly more prominent in preparations from cells grown with fumarate and identified as formate dehydrogenase.

For LC/LC-MS/MS analysis, biotin-labeled surface membrane protein samples from aerobically and anaerobically (with fumarate) grown *S. oneidensis* were digested with trypsin and separated by 2-dimensional chromatography using 12 ammonium acetate salt pulses. 2D-LC-ES-MS/MS was done using a linear ion trap mass spectrometer (Thermo Finnigan LTQ). Datasets were searched with SEQUEST, filtered, and sorted with DTASelect [minimum Xcorrs of 1.8 (+1), 2.5 (+2), 3.5 (+3), DelCN of at least 0.08], and compared with Contrast. Proteins were accepted as identified if at least two fully tryptic peptides passing the above criteria were identified from a given LC-MS/MS analysis. The table shows identifications for the proteins showing the most significant difference in expression in the comparison of MR-1 grown with oxygen or fumarate as the terminal electron acceptor. Formate dehydrogenase is an example of a protein that has been identified by both 2DE/LC/MS-MS and 2DLC/MS-MS as expressed on the surface of cells grown with fumarate but not with oxygen. The different colors of highlighting indicate groupings of differential (yellow, green, orange) or unchanged (blue) protein expression.



Summary

By labeling intact cells with biotinylation reagents reacting with free amino groups, the proteins exposed at the surface of the cells can be enriched using avidin affinity chromatography. Analysis using fluorescent dyes specific for dead and live cells has shown that the reaction conditions used are gentle enough to ensure cell viability and, therefore, minimal exposure of intracellular proteins to the labeling reagent. Using both 2DE and 2DLC/MS-MS, we are revealing differential protein expression at the surface of MR-1 grown with different electron acceptors. Of particular importance are the differences seen when cells are grown with insoluble iron oxides. Protein identifications from cells grown with soluble electron acceptors indicate that proteomics methods will allow the identification of these protein differences.



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